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2. Patent application number *(The Patent Office will fill in this part)* — 23 MAY 2003 ✓ — 0311946.8 ✓

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Sense Proteomic Limited
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Gardner Road, Maidenhead
Berkshire, SL6 7RJ

Patents ADP number *(if you know it)* 07810316001

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

4. Title of the invention — ENZYME ARRAY AND ASSAY

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Stratagem IPM Ltd
Fosters Wing, Anstey Hall
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Trumpington
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CB2 2LF

Patents ADP number *(if you know it)* 07995780002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
United Kingdom	0224872.2	25 Oct 2002
PCT	PCT/EP02/14859	20 Dec 2002

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number or earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

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Claim(s)	4
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11. I / We request the grant of a patent on the basis of this application.

Signature Swatragem IPM Ltd. Date 23 May 03

12. Name and daytime telephone number of person to contact in the United Kingdom: Dominic Schiller 0870 161 1673

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ENZYME ARRAY AND ASSAY

FIELD OF THE INVENTION

The present invention relates to an enzyme array and assay and more particularly to a kinase array and assay for use with a mass spectrometer, particularly, though not exclusively, a laser desorption/ ionisation, such as a MALDI mass spectrometer.

BACKGROUND TO THE INVENTION

Proteomic applications for mass spectrometry have seen a strong growth in recent years. Analytical methods used in proteomics are mainly based on 2D-gel electrophoresis for protein separation, and either mass spectrometry or Edman degradation for protein identification. The limitations of 2D gel electrophoresis include relatively poor resolution, sensitivity and reproducibility. As a result proteomic methods which avoid 2D-gel electrophoresis such as Isotope Coded Affinity Tag (ICAT) [1], Tandem Affinity Protein (TAP) [2] purification and the use of protein microarrays [3] are gaining popularity.

Furthermore, these new methods have broadened the scope of proteomics from collecting and cataloguing differential expression data to a stage where relations between molecules can be assigned and this has been referred to as functional proteomics. Protein microarrays have recently been used to analyze 119 yeast kinases [4]and a major fraction of the yeast proteome [5].

Protein microarrays have been analyzed by enhanced chemi-luminescence (ECL), fluorescent or radioactive labels or via antibody based detection systems, but not to date by mass spectrometry.

The current reliance on the use of labeled ligands, such as antibodies or labeled probes, to analyze protein microarrays imposes constraints on the applications for protein microarrays. Hence a sensitive label free detection system would be of great advantage and would broaden the range of application to areas where labeled compounds either are not available, or are too expensive or where labeling would fundamentally alter the

properties of the ligand. Such a label free method would be particularly useful in the early stages of drug discovery, where great numbers of compounds are screened against proteins.

Such a mass spectrometry probe, upon which an enzyme microarray has been fabricated, enables interrogation of enzymatic reactions and the effect compounds have thereon in a label-free manner by desorption and ionisation of reactants and products. The probe and methods are particularly useful in the drug discovery process, for example in hit series evaluation, lead optimisation, predictive toxicogenomics and metabolite profiling.

The probes and method could however be used as a diagnostic tool to both diagnose disease states and monitor disease progression.

SUMMARY OF THE INVENTION

According to a first aspect of the invention there is provided a method of determining the activity of an enzyme, or the effect a test compound has on the activity of the enzyme, using mass spectrometry comprising:

i) providing a probe carrying an immobilised enzyme;
ii) optionally introducing the test compound;
iii) introducing one or more reactants to the immobilised enzyme for a time, and in a form sufficient for a reaction to take place;
iv) drying the probe;
v) subjecting the probe to mass spectrometry; and
vi) determining the activity of the enzyme, or the effect the test compound had on the activity of the enzyme, by detecting the presence and/ or absence of one or more products and/ or the one or more reactants.

Preferably the mass spectrometry uses a MALDI mass spectrometer. However, since the principal mass spectrometry requirement for analysis is that the molecule be converted to

gaseous ions, the skilled person will appreciate that numerous other mass spectrometry methods which enable this ionisation event can also be used, including without limitation: laser desorption-ionisation mass spectrometry; matrix-assisted laser desorption-ionisation (MALDI) mass spectrometry; desorption-ionisation on silicon (DIOS) mass spectrometry; electrospray ionisation mass spectrometry; and atmospheric pressure ionization (API) mass spectrometry. Clearly fourier transform mass spectrometry (FT/MS) methods such as fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry can be used to enhance the mass accuracy of the various ionisation methods.

For example, in the case of electrospray ionisation methods, enzymes can be immobilised on individual nozzles of a multi-nozzle spraying probe (for example the NanoMate-100 nanoelectrospray probe sold by Advion), the reactions carried out by introducing one or more reactants into each nozzle, introducing the probe into the spectrometer and ionising the reactants and products by electrospray ionisation.

Whilst the method can be used to study any enzymatic reaction where one or more reactants are converted to one or more products and either the reactants and or the products can be discerned using e.g. MALDI mass spectrometry it is particularly suitable as a method for investigating kinases as all kinases use and / or generate a nucleotide tri phosphate (NTP) or a nucleotide di phosphate (NDP) e.g. adenine tri phosphate (ATP) or adenine di phosphate (ADP) which can be readily and distinguishably detected. The skilled person will understand that other nucleotide species such as guanine, uridine and cytosine may also be used, although adenine is preferred due to the fact it provides for a more sensitive assay as detection can be achieved at pico (10^{-12}) Molar levels. Sensitivity is improved using MALDI mass spectrometry due to the enhancing effects of matrix.

In one embodiment step ii) is essential, and the effect the test compound has on the enzymatic activity is determined by comparison with the results obtained where the test compound is absent. The assay may be run on a single array, by running two or more assays in parallel, or by comparison to a standard. The test compound may be added pre, post or most preferably with the one or more reactants.

The method may be used to provide either a qualitive or quantitive result.

Preferably the method will determine the activity of one or more kinases or the effect a test compound has on the activity of one or more kinases by using MALDI mass spectrometry. Thus a kinase array for use in the method may comprise one or more kinases, for example, at least 10, through 25, 50, 75, 100, 200, 300 or more of the some 500 plus kinases of the kineome. These may be arranged on a microarray, each kinase being deposited at a discrete target area.

For a kinase assay the one or more reactants may comprise a phosphate donor, a phosphate acceptor and a divalent cation. The phosphate donor may be a phosphorylated substrate and the phosphate acceptor may be a nucleotide di phosphate (NDP). The simplicity of the method resides in the fact that these reactants are common to all kinase reactions thus enabling a single set of conditions to be applied across a range of kinases. This means the assay is robust and enables it be used for high throughput screening.

Of course it is possible to use discrete as well as generic substrates and examples of kinases and their substrates are shown in Tables 1 and 2, annexed hereto.

In one embodiment the phosphate donor is a nucleotide tri phosphate (NTP) and the phosphate acceptor is a substrate to be phosphorylated. Preferably the divalent cation (M^{2+}) is magnesium or manganese.

In another embodiment the product is a nucleotide tri phosphate or a nucleotide di phosphate, the presence of which is detected. Of course since a typical kinase reaction is reversible the reactants may be the products and vice versa.

Typical reaction:

(Reactants)		(Products)
E.g. Glucose + ATP	→	Glucose 6 phosphate + ADP

$$\text{(Products)} \xleftarrow{\text{Hexokinase/ } Mg^{2+}} \text{(Reactants)}$$

Typically the nucleotide tri phosphate or nucleotide di phosphate are detected as $[NTP]^-$ or $[NDP]^-$ and / or as one or more adduct peaks thereof. The one or more adduct peaks are typically adduct peaks with a monovalent cat ion (M^+) (e.g. Na, K, Li.) depending on the reagents / buffers used. The one or more adduct peaks may, for example, include $[ATP\ M]^-$, $[ATP\ M_2]^-$, and $[ATP\ M_3]^-$ and / or $[ADP\ M]^-$, $[ADP\ M_2]^-$, and $[ADP\ M_3]^-$.

An important factor in being able to achieve good detection is in the selection of a low salt buffer. Preferably the low salt buffer is a "semi volatile buffer" such as, ammonium bicarbonate buffer. Such buffers do not leave a residue on evaporation as they are converted into gases, which in the case of ammonium bicarbonate are ammonia, carbon dioxide and water. Alternatively, since the reaction mix need only be a "low salt" buffer at the point of vaporisation/ionisation it would be possible to use a buffer containing a higher concentration of a semi-volatile salt and then, after the reaction is finished, remove the semi-volatile buffer in vacuo (either in the mass spec vacuum chamber or in an external vacuum chamber). This however is more complex and less desirable.

A further and significant feature of the invention resides in the fact that in a kinase assay the detected products/ reactants are small; typically less than 1000 daltons and consequently the mass spectrometry analysis can be conducted without having to overlay the probe with energy absorbing molecules (matrix). This simplifies and speeds up the procedure as well as saving costs. However, the addition of matrix increases sensitivity.

Where energy absorbing molecules are applied these should be applied to the probe in register with the immobilised enzyme.

The one or more reactants, and if present the test compound, are preferably introduced to the immobilised enzyme in a compartmentalised form, such as in the form of a droplet. Preferably the droplet has a volume of less than 1 microlitre.

Additionally it is preferred that the assay is conducted in a humid environment.

Of course, as well as kinases the method of the invention is applicable to other enzymes. Thus, it is possible to study enzyme reactions of immobilized proteins on protein arrays by mass spectrometry wherever the reactants and / or products are ionisable and where the enzymatic reaction leads to a mass change in the reactant and or product. This can be the case for Oxidoreductases, Transferases, Hydrolases, Lyases and Ligases.

Typical subclasses of enzymes from these groups of enzymes are listed in table 3 below:

Table 3

Subclass	Name
EC1	**Oxidoreductases**
EC 1.1	Acting on the CH-OH group of donors
EC 1.2	Acting on the aldehyde or oxo group of donors
EC 1.3	Acting on the CH-CH group of donors
EC 1.4	Acting on the CH-NH_2 group of donors
EC 1.5	Acting on the CH-NH group of donors
EC 1.6	Acting on NADH or NADPH
EC 1.7	Acting on other nitrogenous compounds as donors
EC 1.8	Acting on a sulfur group of donors
EC 1.9	Acting on a heme group of donors
EC 1.10	Acting on diphenols and related substances as donors
EC 1.11	Acting on a peroxide as acceptor
EC 1.12	Acting on hydrogen as donor
EC 1.13	Acting on single donors with incorporation of molecular oxygen (oxygenases)
EC 1.14	Acting on paired donors, with incorporation or reduction of molecular oxygen
EC 1.15	Acting on superoxide radicals as acceptor
EC 1.16	Oxidising metal ions
EC 1.17	Acting on CH_2 groups
EC 1.18	Acting on iron-sulfur proteins as donors
EC 1.19	Acting on reduced flavodoxin as donor
EC 1.20	Acting on phosphorus or arsenic in donors
EC 1.21	Acting on X-H and Y-H to form an X-Y bond
EC 1.97	Other oxidoreductases
EC2	Transferases

EC 2.1	Transferring one-carbon groups
EC 2.2	Transferring aldehyde or ketonic groups
EC 2.3	Acyltransferases
EC 2.4	Glycosyltransferases
EC 2.5	Transferring alkyl or aryl groups, other than methyl groups
EC 2.6	Transferring nitrogenous groups
EC 2.7	Transferring phosphorus-containing groups
EC 2.8	Transferring sulfur-containing groups
EC 2.9	Transferring selenium-containing groups
EC3	**Hydrolases**
EC 3.1	Acting on ester bonds
EC 3.2	Glycosylases
EC 3.3	Acting on ether bonds
EC 3.4	Acting on peptide bonds (peptidases)
EC 3.5	Acting on carbon-nitrogen bonds, other than peptide bonds
EC 3.6	Acting on acid anhydrides
EC 3.7	Acting on carbon-carbon bonds
EC 3.8	Acting on halide bonds
EC 3.9	Acting on phosphorus-nitrogen bonds
EC 3.10	Acting on sulfur-nitrogen bonds
EC 3.11	Acting on carbon-phosphorus bonds
EC 3.12	Acting on sulfur-sulfur bonds
EC 3.13	Acting on carbon-sulfur bonds
EC 4	**Lyases**
EC 4.1	Carbon-carbon lyases
EC 4.2	Carbon-oxygen lyases
EC 4.3	Carbon-nitrogen lyases
EC 4.4	Carbon-sulfur lyases
EC 4.5	Carbon-halide lyases
EC 4.6	Phosphorus-oxygen lyases
EC 4.99	Other lyases
EC 6	**Ligases**
EC 6.1	Forming carbon—oxygen bonds
EC 6.2	Forming carbon—sulfur bonds
EC 6.3	Forming carbon—nitrogen bonds

EC 6.4	Forming carbon—carbon bonds
EC 6.5	Forming phosphoric ester bonds

Alternatively the method can be used to monitor enzymatic reactions involving co-substrates (also reactants in the context of this application) including NAD, NADP, NADH, NADPH, ATP, GTP, UTP, CTP, UDP-glucose, UDP-glucosamine, UDP-galactose, pyridoxalphosphate, UDP-*N*-acetyl-D-glucosamine, GDP-D-mannose, dTDP-6-deoxy-L-mannose, GDP-6-deoxy-D-talose, UDP-*N*-acetylmuramate, *S*)-3-hydroxyacyl-CoA, *S*-adenosyl-L-methionine, acetyl-CoA, L-selenoseryl-tRNA^{Sec}, (*S*)-3-hydroxy-3-methylglutaryl-CoA, 5,10-methylentetrahydrofolate, ascorbate, 2-oxoglutarate, glutathione, pyruvate and tetrahydropteridine.

This is particularly useful when the substrates or products are not ionisable or when the reaction does not cause a mass change, as is seen for Isomerases (for example phenylalanine racemase which is ATP-hydrolysing). Typical isomerases are listed in table 4.

Table 4.

EC6	Isomerases
EC 5.1	Racemases and epimerases
EC 5.2	*cis-trans*-Isomerases
EC 5.3	Intramolecular isomerases
EC 5.4	Intramolecular transferases (mutases)
EC 5.5	Intramolecular lyases
EC 5.99	Other isomerases

More specifically, the enzyme is drawn from one or more of the group or groups of those enzyme families that are common drug targets, such as protein kinases (including serine/threonine kinases and tyrosine kinases), proteases (including serine proteases, cysteine proteases, aspartyl proteases and metalloproteinases), carboxylases, esterases, phosphodiesterases, protein phosphatases (including tyrosine phosphatases), G-protein coupled receptors, ATP-dependent chaperones, cyclooxygenases, cytochrome P450s, sialidases, and short-chain dehydrogenases/reductases.

According to a further aspect of the present invention there is provided a probe for use with a mass spectrometer, comprising a support having an electroconductive target surface thereon characterised in that the target surface comprises an array having a plurality of enzymes immobilised thereon.

In a preferred embodiment the enzymes are selected from the groups of enzymes listed above. Particularly preferred are those enzyme families that are common drug targets, particularly though not exclusively kinases.

Preferably the array is a micro array.

The enzymes are preferably attached to the probe as fusion proteins, typically via a tag, such as, for example, biotin, or a sh ble protein.

Related aspects to the invention are described in full in a number of the applicant's earlier patent applications including WO 01/57198 and are thus not dealt with in depth herein, but as others are as yet unpublished, such as GB 0224872.2, further supporting and related details are given below:

Thus, the probes referred to herein include microarrays, as well as arrays in which the protein spots will be visible to the naked eye, and are adapted so that they may be interrogated by means of laser desorption/ ionisation mass spectrometry, particularly, though not exclusively, matrix assisted laser desorption/ ionisation (MALDI).

Additional aspects relevant to the invention include methods leading to the production of such a probe which can be interrogated by means of laser desorption/ ionisation mass spectrometry, particularly matrix assisted laser desorption/ionisation (MALDI) and methods of analysing such a probe or protein microarray.

Thus, the development of a MALDI MS-compatible protein microarray which term includes enzyme microarrays was complex since existing methods of forming protein microarrays did not transfer readily to a MALDI target. There are a number of reasons why this was the case, including the specialised nature of the probe surfaces and the potential for salts present in reaction buffers to interfere with the detection method.

Procedures known in the art for MALDI typically require the co-crystallization of the aqueous analyte with acidic energy absorbing molecules, or 'matrix', to promote ionization of the analytes (Karas and Hillenkamp, 1988). The method of co-crystallizing analyte and matrix for MALDI, as known in the art, typically results in a heterogeneous crystallization process and yields discrete, spatially separated crystals that each contains differing amounts of matrix and analyte. As a consequence it is often observed that individual crystals contain insufficient analyte for analysis by MALDI. This in turn results in a requirement for the analyser to sample multiple (i.e. 10-100 or more) discrete locations within a given target area in order to obtain a good analyte signal; this is sometime referred to as "the search for the sweet spot". This has previously prevented miniaturization since protein spots needed to be large. They were generally in the order of at least $0.5mm^2$

In order to generate MALDI MS-compatible protein microarrays, solutions for the aforementioned shortcomings of the prior art were required that enabled both miniaturization of the target areas and functional analysis of the arrayed proteins.

As defined herein a probe is a support which is capable of acting as a target in analysis by laser desorption/ionisation mass spectrometry, for example matrix assisted laser desorption/ionisation (MALDI). The probe carries the enzymes, e.g. kinases and the reactants (and optionally test compounds) are added. After a time sufficient for a reaction to take place, and products to be formed, the probes are dried and subjected to mass spectrometry. The reactants and or the products interact with the repeller lens of the ion-optic assembly found in laser desorption/ionisation time-of-flight (TOF) mass spectrometers of the art, such that the converted to gaseous ions which permits analysis. For example, the probes of the invention may be derived from targets for MALDI analysis as known in the art, which are treated such that a high affinity protein binding moiety e.g. streptavidin, avidin or neutravidin molecules are present on the probe surface which bind biotinylated enzymes for subsequent analysis. For example, conventional glass or gold MALDI targets may be used.

As defined herein a micro array is an array where the size of the discrete target areas i.e. the individual areas probed by a laser, is in the order of micrometers or less. Whilst at the upper end of the scale, around 1000 micrometers diameter, they may be visible to the naked eye at the lower end of the scale the discrete target areas will not be clearly distinguished by the naked eye.

The arrays will typically be arranged in matrices comprising several rows and columns. The number of discrete target areas will depend upon what is being screened though it is generally desirable to have a high density of these discrete areas on the probe surface as this will facilitate high through put screening. Typically a probe will comprise at least 10, more preferably at least 100, more preferably at least 1000 and as many as 10,000 or more target areas produced thereon. (Typically a probe surface will have an area of around 10,000mm^2 – a Bruker probe has an area of 10,292mm^2 although there is no requirement to use the whole of the probe and the microarray can be applied in one or more matrices thereon.) The actual density in a given matrices will depend upon the size of the discrete target area (which will typically be printed as a spot) and the spacing between adjacent spots. Thus the discrete target areas will typically be present at a density of greater than 1 discrete target areas per mm^2 within any matrices.

The enzyme is the moiety about which the reaction occurs.

The term "enzyme" as used herein is used to include both whole enzymes and sub units or domains thereof.

"Fusion protein" as used herein is used to refer to an enzyme, which has a tag, for example, a biotinylation consensus sequence or phleomycin/zeocin resistance binding protein attached thereto.

"Linker molecules" are molecules which function as their name suggests. They are molecules comprising functional groups which allow bridges to be formed between different molecules.

Another significant development enabling the "miniaturization" of an enzyme array formed on a MALDI target derives from the application of the Applicant's COVET technology described in WO 01/57198. Briefly, using this technology they are able to create from cDNA libraries expressed enzymes, which carry a "sequence tag" that can be used to capture the enzymes with a high affinity and in a specific orientation on the microarray surface. This firstly enables enzymes e.g. a kinase library to be stably immobilized such that leaching of enzymes from the surface is avoided and secondly the oriented immobilization of the fusion protein onto the surface ensure maximum biological activity.

Yet another significant aspect of the invention, when compared to current protein microarrays, is the provision of such a probe with an electro conductive surface. This surface which includes semi conductive surfaces is essential where the probe is to be subjected to MALDI MS analysis. Whilst the support could be made wholly of an electro conductive material (which term is used herein to include semi conducive materials) it is preferred to coat a rigid support, e.g. a glass, with an electro conductive material such as, for example, gold although any suitable metal, for example, silver, platinum, iridium, wolfram, copper, cobalt, nickel, and iron or mixtures thereof, or a semiconductor e.g. silicon oxide, graphite or germanium oxide could be used.

Where the probe or enzyme microarray is produced on e.g. a standard size microscope glass slide it can be mounted in an adapter, which carries it into a mass spectrometer. Such an adaptor is described in Applicant's co pending UK application no. 0216387.1.

A further significant development, and one which may be viewed independently of the specific applications described herein, has been in the way the Applicant has overcome the problems caused by non specific protein binding. The Applicant has overcome this particular problem by providing a layer resistant to non specific protein binding onto the probe surface. More particularly, the microarray surface is modified by the inclusion of a layer of molecules which repel proteins. These protein repellant molecules which include,

for example, polyethylene glycol may be bound to the probe surface via a linker, such as, for example, a poly amino acid which readily binds to e.g. a glass or gold surface and whose amino or carboxyl side groups can be used to bind the protein repellant molecules such that they reach out from the probe surface. The skilled man will appreciate that other functionalized molecules could be used. Preferably the enzyme binding moieties are incorporated in a position where they extend out from the surface. Preferred enzyme binding moieties include e.g. biotin, biotin-neutravidin, and bleomycin, and these and other moieties can be incorporated into the layer either via these functional groups on the linker molecules and/ or via functional groups on the protein repellant molecules. Typically the affinity capture moieties are incorporated in small proportions (typically less than 20%) relative to the protein repellant molecules.

In this way the Applicant has been able to introduce the enzyme capture moieties not only in a homogeneous, spatial defined arrangement but also in a manner which enables high affinity binding in a specific manner. The resulting surface combines selectivity for the capture of biological macromolecules on the probe with reduced non specific binding of the type commonly observed on underivatised glass or metal surfaces and additionally results in a homogeneous distribution and orientation of the captured biological macromolecules.

The component molecules responsible for repelling non specific proteins include molecules which are generally hydrophilic in nature. They include polymers, such as, for example, polyethylene glycol, dextran, polyurethane and polyacrylamide and self assembled monolayers (SAM). Preferably the polymers comprise one or more functional side groups via which the protein capturing moieties can be attached. In the case of polyethylene glycol the functional group is a hydroxyl group. The molecules responsible for repelling non specific proteins may be bound directly to the surface as in, for example the case of SAM's or they may be attached via a linker. Particularly preferred as linkers are poly amino acids such as, for example, poly L lysine, poly L aspartic acid, poly L glutamic acid or mixtures thereof. These have amino or carboxy side chains via which the molecules responsible for repelling non specific proteins can be attached and which

can additionally be used to attach the protein capturing moieties. Alternatively, or in addition, the protein capturing moieties can be attached via the component molecules responsible for repelling non specific proteins.

Fig 1 illustrates the binding of such molecules and contrasts the defined orientation which can be achieved by this ordered coupling compared to that achieved using current antibody binding techniques which result in random coupling.

In a preferred embodiment the probe has as it's enzyme capture moieties either a biotin binder e.g. neutravidin, avidin or streptavidin or a bleomycin resistant protein binder e.g. bleomycin. The enzymes are bound to the probe to create a protein microarray by printing a plurality of bacterial, yeast, sf9 or mammalian cell lysates containing fusion proteins in which a high affinity tag e.g. biotin or zeocin resistant protein (ZRP) is expressed onto the capture surface. Proteins are derived from the expression of a cDNA library and each individual clone is tagged at the C-terminus and/ or on the N-terminus with a consensus sequence, which will enable high affinity recognition of the enzyme even in the presence of the otherwise protein repellent molecules. Only the recombinant, tagged enzyme can recognise the capture surface and other proteins from the lysate can be washed away as they do not bind to the protein repellent surface and do not have a high affinity to the protein binding moieties present in the layer.

A further aspect of the present invention provides a method of producing an enzyme microarray for use with a mass spectrometer comprising providing a probe of the invention and depositing the enzyme in registration with the protein capturing moieties in the discrete target area.

Yet a further aspect of the present invention provides a method of analyzing a probe of the invention in which energy absorbing molecules are deposited in a manner which denatures and thus unbinds an enzyme from a protein capturing moiety leaving the denatured enzyme lying unbound on the surface.

P1660

The energy absorbing molecules form a homogenous layer of crystals in a discrete location in registration with the protein capturing moieties and captured enzyme.

The homogenous layer of crystals is substantially continuous such that individual crystals are not visible at a 100 fold magnification and there are no visible gaps. It also has a substantially uniform depth, such that there is no apparent variation in crystal size at a 100 fold magnification.

The energy absorbing molecules are deposited onto the surface in a non aqueous solvent and the non aqueous solvent is evaporated off. Preferably the non aqueous solvent is an organic solvent, such as, for example, acetone or butanone. Preferably the non aqueous solvent includes a modifier which controls the rate of evaporation such that crystallization of the energy absorbing molecules occurs on the probe. Suitable modifiers include glycerol, polyethylene glycol and thioglycerol. Preferably the energy absorbing molecules are deposited in a mixture of from 80 - 99.9%, preferably 99% organic solvent e.g. acetone to 20 - 0.1%, preferably 1% of modifier e.g. glycerol (vol/vol) .Typical energy absorbing molecules include crystals of α-cyano-4-hydroxy-cinnamic acid, sinapinic acid, gentisic acid, nifidine, succinic acid, 1,8,9,-anthracenitriol, 3-Indoleacrylic acid, 2-(hydroxyphenylazo) benzoe-acid, 4-nitroanilin and combinations thereof.

Preferably the energy absorbing molecules are deposited in registration with the protein and each protein spot is overlaid with a similar sized spot of the energy absorbing molecules.

In order to achieve a high density of individual samples on the microarray the energy absorbing molecules need to be arranged in microcrystals on the surface. The matrix forms a homogenous layer of flat crystals without significant gaps between them and can be deposited in very small quantities on the microarray.

In contrast to the prior art in which matrix and analyte are co crystallized in an aqueous solvent, the Applicant uses two distinct steps in which first the protein is deposited in an aqueous solvent and then the energy absorbing molecules are deposited such that they crystallise out from the non aqueous solvent on the probe. This has the advantage that the protein is deposited in its biological form. However, using a non aqueous solvent to deliver the energy absorbing molecules allows the formation of a homogenous layer of microcrystals. This has two benefits. First the formation of a homogenous layer means it is not necessary to search for a sweet spot as the homogenous layer guarantees protein in the presence of energy absorbing molecules and secondly it results in more accurate measurement due to the even nature of the layer.

BRIEF DESCRIPTION OF THE DRAWING

The various aspects of the invention will now be described, by way of example only, with reference to the following figures and examples in which:

Fig 1 shows the orientated binding of the enzymes to a probe
Fig 2, shows the detection of ADP and ATP using mass spectrometry, and
Fig 3 also shows creatine phosphate mediated ATP synthesis on a surface

DETAILED DESCRIPTION

EXAMPLE 1

Referring to Fig 2, ATP was enzymatically synthesized from the reaction of ADP, creatine phosphate and creatine kinase (also known as creatine phosphokinase) in 25 mM ammonium bicarbonate at pH 7.4. $[ADP]^-$ was detected at 427.6 dalton and $[ADP+Na]^-$ at 449.6 dalton. The products of the creatine phosphate kinase reaction were detected at 507.6, 529.6, 551.6 and 573.8, which fit well with the expected molecular weight of $[ATP]^-$ and three ATP sodium adducts $[ATP\ Na]^-$. $[ATP\ Na_2]^-$ and $[ATP\ Na_3]^-$.

Control reactions in which either one of the substrates ADP or creatine phosphate or the enzyme creatine phosphate kinase was omitted didn't show ATP peaks.

EXAMPLE 2

Referring to Fig 3 the example demonstrates the biotinylation, capture, and desalting of creatine kinase from rabbit muscle on a PEG-PLL-Biotin-Neutravidin coated probe for analysis by MALDI mass spectrometry and the enzymatic activity of the immobilized kinase using MALDI mass spectrometry.

Material:

Creatine kinase from rabbit muscle ATP: creatine N-phosphotransferase
ADP, creatine phosphate, 1 mM Tris HCl pH 7.5, 1 mM $MgCl_2$, gold coated glass slide, PEG-PLL-biotin, Neutravidin

Solutions:

Washing buffer: 1 mM Tris-HCl pH 7.5 with 0.1% Triton X-100; desalting buffer: 1 mM Tris-HCl pH 7.5.

Affinity capture polymer synthesis

The poly-L-lysine PEG-biotin (PEG-PLL-biotin) was synthesized according to the protocol of Ruiz Taylor [6]. Briefly, 100 mg poly-L-lysine (average size 17-30 kDa; Sigma, Dorset, UK) was reacted with 109 mg mPEG-SPA and 1.1 mg biotinPEG-CO-NHS (Shearwater Corporation, Huntsville, Alabama) in 1ml 100 mM sodium carbonate buffer pH 9 for a period of 30 minutes. The reaction was terminated by dialysis in 1 mM Tris-HCl pH 7.5 overnight. The product from this reaction was called PEG-PLL-biotin (1% PEG derivatives contain a biotin headgroup).

Biotinylation of Creatine kinase

Creatine kinase (100 mg) was dissolved in 1 ml 1 mM Tris HCl pH 7.5 and 1mg of EZ link biotin PEO amine and 1 mg ethylene diamine carbodiimide were reacted for 20 minutes at room temperature.

Affinity capture surface preparation

Protein microarray probes were thoroughly cleaned before use with sequential washing steps in acetone, acetonitrile, double distilled water and dried under nitrogen. Freshly prepared affinity capture polymer PEG-PLL-Biotin was then pipetted on the surface of the probe and was then evenly distributed on the surface by covering it with Nesco film (Azwell Inc., Osaka, Japan). After 30 min the probe was washed in 1 mM Tris-HCl pH 7.5, dried under nitrogen and then coated with 0.5 mg/ml neutravidin for one hour at RT in a humid chamber. The probe was then rinsed with washing buffer, washed twice with desalting buffer and dried under nitrogen. The surface was now ready to be used as a highly specific affinity capture device for biotinylated macromolecules.

Capture and detection of biotinylated proteins on the probe surface

A PLL-PEG-biotin neutravidin surface on a MALDI target was overlaid with 50 ng of biotinylated creatine kinase (Roche, Mannheim, Ger) The biotinylated protein was captured for a period of 2 hours on the MALDI target in a humid chamber to prevent drying, washed twice with washing buffer followed by two washes with desalting buffer, surface was dried under nitrogen and overlaid with 300 nl of a saturated solution of cyano-4-hydroxycinnamic acid in acetone.

Monitoring the kinase activity of the immobilized Creatine Kinase on the protein array

The array with the immobilized creatine kinase is washed with 100 ml 1 mM Tris-HCl pH 7.5 and is then overlaid with a mixture 1 mM creatine phosphate, 1 mM ADP, 1 mM MgCl2 and 25 mM ammoniumbicarbonate buffer in a volume less than 1 microlitre. The enzyme and the substrates are incubated in a humid chamber at 37°C for a period of 30 minutes. Reactions which omitted either ADP, creatine phosphate or the kinase were run in parallel as specificity controls.

Results

Fig 3 shows the detection of ADP and ATP. ATP was enzymatically synthesized from the reaction of ADP, creatine phosphate and creatine kinase in 25 mM ammonium bicarbonate at pH 7.4. $[ADP]^-$ was detected at 427.6 dalton and $[ADP+Na]^-$ 449.6 dalton. The products of the creatine kinase reaction were detected at 507.6, 529.6, 551.6 and 573.8, which fits well with the expected molecular weight of $[ATP]^-$ and three ATP sodium adducts [ATP Na] . $[ATP\ Na_2]^-$ and $[ATP\ Na_3]^-$.

Control reactions in which either one of the substrates ADP or creatine phosphate or the enzyme creatine kinase was omitted didn't show ATP peaks.

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CLAIMS

1. A method of determining the activity of an enzyme, or the effect a test compound has on the activity of the enzyme, by using mass spectrometry comprising:

i) providing a probe carrying an immobilised enzyme;

ii) optionally introducing the test compound;

iii) introducing one or more reactants to the immobilised enzyme for a time, and in a form sufficient for a reaction to take place;

iv) drying the probe;

v) subjecting the probe to mass spectrometry; and

vi) determining the activity of the enzyme, or the effect the test compound had on the activity of the enzyme, by detecting the presence and/ or absence of one or more products and/ or the one or more reactants.

2. A method as claimed in claim 1 wherein the mass spectrometry is a laser desorpton ionisation mass spectrometry, preferably a MALDI mass spectrometry.

3. A method as claimed in claim 1, 2 or 3 wherein the enzyme is a kinase, oxidoreductase, transferase, hydrolase, lyase, or ligase.

4. A method as claimed in claim 1 or 2 wherein the enzyme is a protein kinase, protease, carboxylase, esterase, phosphodiesterase, protein phosphatase, G-protein coupled receptor, ATP-dependent chaperone, cyclooxygenase, cytochrome P450, sialidase, short-chain dehydrogenase or short-chain reductase.

5. A method as claimed in claim 4 wherein the enzyme is a serine kinase, threonine kinase, tyrosine kinase, serine protease, cysteine protease, aspartyl protease, metalloproteinase or tyrosine phosphatase,

6. A method as claimed in any of the preceding claims wherein step ii) is essential, and the effect the test compound has on the enzymatic activity is determined by comparison to the results obtained in its absence.

7. A method as claimed in claim 6 which comprises adding the test compound pre, post or with the one or more reactants to determine its effect on enzyme activity.

8. A method as claimed in any of the preceding claims which is qualitive.

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9. A method as claimed in any of claims 1 to 7 which is quantitive.

10. A method of determining the activity of a kinase or the effect a test compound has on the activity of a kinase by using mass spectrometry comprising:

i) providing a probe carrying an immobilised kinase;

ii) optionally introducing the test compound;

iii) introducing one or more reactants to the immobilised kinase for a time, and in a form sufficient for a reaction to take place;

iv) drying the probe;

v) subjecting the probe to mass spectrometry; and

vi) determining the activity of the kinase or the effect the test compound has on the activity of the kinase by detecting the presence and/ or absence of one or more products and /or the one or more reactants.

11. A method as claimed in claim 10 wherein the mass spectrometry is a laser desorpton ionisation mass spectrometry, preferably a MALDI mass spectrometry.

12. A method as claimed in any of claims 3 to 11 wherein the one or more reactants comprise a phosphate donor, a phosphate acceptor and a divalent cation.

13. A method as claimed in claim 12 wherein the phosphate donor is a phosphorylated substrate and the phosphate acceptor is a nucleotide di phosphate (NDP).

14. A method as claimed in claim 12 wherein the phosphate donor is a nucleotide tri phosphate (NTP) and the phosphate acceptor is a substrate to be phosphorylated.

15. A method as claimed in claim 12 wherein the divalent cation is magnesium or manganese.

16. A method as claimed in claim 13 or 14 wherein the nucleotide di phosphate or tri phosphate is an adenine di or tri phosphate (ADP or ATP).

17. A method as claimed in any of the preceding claims wherein the product is a nucleotide tri phosphate or a nucleotide di phosphate and its presence is detected.

18. A method as claimed in claim 17 wherein the nucleotide tri phosphate or nucleotide di phosphate are detected as $[NTP]^-$ or $[NDP]^-$ or as one or more adduct peaks thereof.

19. A method as claimed in claim 18 wherein the one or more adduct peaks are adduct peaks with a monovalent cat ion (M^+).

20. A method as claimed in claim 19 wherein the one or more adduct peaks include: [ATP M]$^-$, [ATP M_2]$^-$, and [ATP M_3]$^-$ and / or [ADP M]$^-$, [ADP M_2]$^-$, and [ADP M_3]$^-$.

21. A method as claimed in any of claims which further comprises, between step iv) and v), overlaying the probe with energy absorbing molecules.

22. A method as claimed in any of the preceding claims wherein the one or more reactants, and if present the test compound, are introduced to the immobilised enzyme in a compartmentalised form.

23 A method as claimed in claim 22 wherein the compartmentalised form is as a droplet.

24. A method as claimed in claim 23 wherein the droplet has a volume of less than 1 microlitre.

25. A method as claimed in any of the preceding claims wherein the one or more reactants are provided in a low salt buffer.

26. A method as claimed in claim 25 wherein the low salt buffer is an ammonium bicarbonate buffer.

27. A method as claimed in any of the preceding claims wherein the assay is conducted in a humid environment.

28. A method as claimed in any of the preceding claims wherein the one or more reactants and optionally any energy absorbing molecules are applied to the probe in register with the immobilised enzyme.

29. A probe for use with a mass spectrometer, comprising a support having an electroconductive target surface thereon characterised in that the target surface comprises an array having a plurality of enzymes immobilised thereon.

30. A probe as claimed in claim 29 wherein the enzymes are kinases, oxidoreductases, transferases, hydrolases, lyases, or ligases.

31. A probe as claimed in claim 29 or 30 wherein the enzymes are protein kinases, proteases, carboxylases, esterases, phosphodiesterases, protein phosphatases, G-protein coupled receptors, ATP-dependent chaperones, cyclooxygenases, cytochrome P450's, sialidases, short-chain dehydrogenases or short-chain reductases.

32. A probe as claimed in claim 31 wherein the enzyme is a serine kinase, threonine kinase, tyrosine kinase, serine protease, cysteine protease, aspartyl protease, metalloproteinase or tyrosine phosphatase,

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33. A probe as claimed in any of claims 29 to 32 wherein the array is a micro array.

34. A probe as claimed in any of claims 29 to 33 wherein the enzyme is a fusion protein.

35. A probe as claimed in any of claims 29 to 34 wherein the enzyme is immobilised via a tag.

36. A probe as claimed in claim 35 wherein the tag is a biotin or a ble protein.

Table 1.

SWISS-PROT Accession Number	Protein Name
P43403	TYROSINE-PROTEIN KINASE ZAP-70 (70 KDA ZETA-ASSOCIATED PROTEIN) (SYK-RELATED TYROSINE KINASE)
P07947	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE YES (P61-YES) (C-YES)
O60285	PROBABLE SERINE/THREONINE-PROTEIN KINASE KIAA0537
P30291	WEE1-LIKE PROTEIN KINASE (WEE1HU)
P35916	VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 3 PRECURSOR (VEGFR-3) (TYROSINE-PROTEIN KINASE RECEPTOR FLT4)
P17948	VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 1 PRECURSOR (VEGFR-1) (TYROSINE-PROTEIN KINASE RECEPTOR FLT) (FLT-1) (TYROSINE-PROTEIN KINASE FRT)
O75385	SERINE/THREONINE-PROTEIN KINASE ULK1 (UNC-51-LIKE KINASE 1)
P30530	TYROSINE-PROTEIN KINASE RECEPTOR UFO PRECURSOR (AXL ONCOGENE)
Q06418	TYROSINE-PROTEIN KINASE RECEPTOR TYRO3 PRECURSOR (TYROSINE-PROTEIN KINASE RSE) (TYROSINE-PROTEIN KINASE SKY) (TYROSINE-PROTEIN KINASE DTK)
P29597	NON-RECEPTOR TYROSINE-PROTEIN KINASE TYK2
O43914	TYRO PROTEIN TYROSINE KINASE-BINDING PROTEIN PRECURSOR (DNAX-ACTIVATION PROTEIN 12)
P42681	TYROSINE-PROTEIN KINASE TXK
P33981	DUAL SPECIFICITY PROTEIN KINASE TTK (PYT)
P04629	HIGH AFFINITY NERVE GROWTH FACTOR RECEPTOR PRECURSOR (TRK1 TRANSFORMING TYROSINE KINASE PROTEIN) (P140-TRKA) (TRK-A)
Q02763	ANGIOPOIETIN 1 RECEPTOR PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR TIE-2) (TYROSINE-PROTEIN KINASE RECEPTOR TEK) (P140 TEK) (TUNICA INTERNA ENDOTHELIAL CELL KINASE)
P35590	TYROSINE-PROTEIN KINASE RECEPTOR TIE-1 PRECURSOR
P36897	TGF-BETA RECEPTOR TYPE I PRECURSOR (TGFR-1) (TGF-BETA TYPE I RECEPTOR) (SERINE/THREONINE-PROTEIN KINASE RECEPTOR R4) (SKR4) (ACTIVIN RECEPTOR-LIKE KINASE 5) (ALK-5)
Q15569	TESTIS-SPECIFIC PROTEIN KINASE 1
P42680	TYROSINE-PROTEIN KINASE TEC
O76039	SERINE/THREONINE-PROTEIN KINASE 9
Q13043	SERINE/THREONINE PROTEIN KINASE 4 (STE20-LIKE KINASE MST1) (MST-1) (MAMMALIAN STE20-LIKE PROTEIN KINASE 1) (SERINE/THREONINE PROTEIN KINASE KRS-2)
Q13188	SERINE/THREONINE PROTEIN KINASE 3 (STE20-LIKE KINASE MST2) (MST-2) (MAMMALIAN STE20-LIKE PROTEIN KINASE 2) (SERINE/THREONINE PROTEIN KINASE KRS-1)
P51957	SERINE/THREONINE PROTEIN KINASE 2 (SERINE/THREONINE-PROTEIN

[redacted]	[redacted]
	KINASE NRK2)
O00506	SERINE/THREONINE PROTEIN KINASE 25 (STERILE 20/OXIDANT STRESS-RESPONSE KINASE 1) (STE20/OXIDANT STRESS RESPONSE KINASE-1) (SOK-1) (STE20-LIKE KINASE)
Q9Y6E0	SERINE/THREONINE PROTEIN KINASE 24 (STE20-LIKE KINASE MST3) (MST-3) (MAMMALIAN STE20-LIKE PROTEIN KINASE 3)
Q9UPE1	SERINE/THREONINE PROTEIN KINASE 23 (MUSCLE-SPECIFIC SERINE KINASE 1) (MSSK-1)
O75716	SERINE/THREONINE PROTEIN KINASE 16 (PROTEIN KINASE PKL12) (MYRISTOYLATED AND PALMITOYLATED SERINE-THREONINE KINASE) (MPSK) (TGF-BETA STIMULATED FACTOR 1) (TSF-1) (HPSK)
Q15831	SERINE/THREONINE-PROTEIN KINASE 11 (SERINE/THREONINE-PROTEIN KINASE LKB1)
O94804	SERINE/THREONINE-PROTEIN KINASE 10 (LYMPHOCYTE-ORIENTED KINASE)
P12931	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE SRC (P60-SRC) (C-SRC)
Q99611	SELENIDE,WATER DIKINASE 2 (SELENOPHOSPHATE SYNTHETASE 2) (SELENIUM DONOR PROTEIN 2)
P49903	SELENIDE,WATER DIKINASE 1 (SELENOPHOSPHATE SYNTHETASE 1) (SELENIUM DONOR PROTEIN 1)
Q9UEW8	STE20/SPS1-RELATED PROLINE-ALANINE RICH PROTEIN KINASE (STE-20-RELATED KINASE) (DCHT)
Q9NYY3	SERINE/THREONINE-PROTEIN KINASE SNK (SERUM INDUCIBLE KINASE)
P57059	PROBABLE SERINE/THREONINE PROTEIN KINASE SNF1LK
Q9BPZ7	STRESS-ACTIVATED MAP KINASE INTERACTING PROTEIN 1 (SAPK INTERACTING PROTEIN 1) (PUTATIVE RAS INHIBITOR JC310)
O00141	SERINE/THREONINE-PROTEIN KINASE SGK (SERUM/GLUCOCORTICOID-REGULATED KINASE)
O94768	SERINE/THREONINE KINASE 17B (DAP KINASE-RELATED APOPTOSIS-INDUCING PROTEIN KINASE 2)
Q9UEE5	SERINE/THREONINE KINASE 17A (DAP KINASE-RELATED APOPTOSIS-INDUCING PROTEIN KINASE 1)
P34925	TYROSINE-PROTEIN KINASE RYK PRECURSOR
Q01974	TYROSINE-PROTEIN KINASE TRANSMEMBRANE RECEPTOR ROR2 PRECURSOR (NEUROTROPHIC TYROSINE KINASE, RECEPTOR-RELATED 2)
Q13516	PROTEIN KINASE C-BINDING PROTEIN RACK17 (PROTEIN KINASE C BINDING PROTEIN 2)
Q13546	SERINE/THREONINE PROTEIN KINASE RIP (CELL DEATH PROTEIN RIP) (RECEPTOR INTERACTING PROTEIN)
Q13308	TYROSINE-PROTEIN KINASE-LIKE 7 PRECURSOR (COLON CARCINOMA KINASE-4) (CCK-4)
Q13882	TYROSINE-PROTEIN KINASE 6 (BREAST TUMOR KINASE) (TYROSINE-PROTEIN KINASE BRK)
P78527	DNA-DEPENDENT PROTEIN KINASE CATALYTIC SUBUNIT (DNA-PKCS) (DNPK1)

[illegible]	[illegible]
Q13523	SERINE/THREONINE-PROTEIN KINASE PRP4 HOMOLOG
P53350	SERINE/THREONINE-PROTEIN KINASE PLK (PLK-1) (SERINE-THREONINE PROTEIN KINASE 13) (STPK13)
P51817	PROTEIN KINASE PKX1
Q16513	PROTEIN KINASE C-LIKE 2 (PROTEIN-KINASE C-RELATED KINASE 2)
Q16512	PROTEIN KINASE C-LIKE 1 (PROTEIN-KINASE C-RELATED KINASE 1) (PROTEIN KINASE C-LIKE PKN) (SERINE-THREONINE PROTEIN KINASE N)
P00558	PHOSPHOGLYCERATE KINASE 1 (PRIMER RECOGNITION PROTEIN 2) (PRP 2)
Q9P286	SERINE/THREONINE-PROTEIN KINASE PAK 5 (P21-ACTIVATED KINASE 5) (PAK-5)
O75914	SERINE/THREONINE-PROTEIN KINASE PAK 3 (P21-ACTIVATED KINASE 3) (PAK-3) (BETA-PAK)
Q13177	SERINE/THREONINE-PROTEIN KINASE PAK 2 (P21-ACTIVATED KINASE 2) (PAK-2) (PAK65) (GAMMA-PAK) (S6/H4 KINASE)
Q13153	SERINE/THREONINE-PROTEIN KINASE PAK 1 (P21-ACTIVATED KINASE 1) (PAK-1) (P65-PAK) (ALPHA-PAK)
O43422	52 KDA REPRESSOR OF THE INHIBITOR OF THE PROTEIN KINASE (P58IPK-INTERACTING PROTEIN) (58 KDA INTERFERON-INDUCED PROTEIN KINASE-INTERACTING PROTEIN) (P52RIPK) (DEATH ASSOCIATED PROTEIN 4)
Q99435	PROTEIN KINASE C-BINDING PROTEIN NELL2 PRECURSOR (NEL-LIKE PROTEIN 2) (NEL-RELATED PROTEIN 2)
Q92832	PROTEIN KINASE C-BINDING PROTEIN NELL1 PRECURSOR (NEL-LIKE PROTEIN 1) (NEL-RELATED PROTEIN 1)
P51956	SERINE/THREONINE-PROTEIN KINASE NEK3 (NIMA-RELATED PROTEIN KINASE 3) (HSPK 36)
P51955	SERINE/THREONINE-PROTEIN KINASE NEK2 (NIMA-RELATED PROTEIN KINASE 2) (NIMA-LIKE PROTEIN KINASE 1) (HSPK 21)
P15531	NUCLEOSIDE DIPHOSPHATE KINASE A (NDK A) (NDP KINASE A) (TUMOR METASTATIC PROCESS-ASSOCIATED PROTEIN) (METASTASIS INHIBITION FACTOR NM23) (NM23-H1)
Q9ULX6	NEIGHBOR OF A-KINASE ANCHORING PROTEIN 95 (HOMOLOGOUS TO AKAP95 PROTEIN) (HA95) (HELICASE A-BINDING PROTEIN 95) (HAP95)
Q13163	DUAL SPECIFICITY MITOGEN-ACTIVATED PROTEIN KINASE KINASE 5 (MAP KINASE KINASE 5) (MAPKK 5) (MAPK/ERK KINASE 5)
P45985	DUAL SPECIFICITY MITOGEN-ACTIVATED PROTEIN KINASE KINASE 4 (MAP KINASE KINASE 4) (JNK ACTIVATING KINASE 1) (C-JUN N-TERMINAL KINASE KINASE 1) (JNKK) (SAPK/ERK KINASE 1) (SEK1)
P46734	DUAL SPECIFICITY MITOGEN-ACTIVATED PROTEIN KINASE KINASE 3 (MAP KINASE KINASE 3) (MAPKK 3) (MAPK/ERK KINASE 3)
P36507	DUAL SPECIFICITY MITOGEN-ACTIVATED PROTEIN KINASE KINASE 2 (MAP KINASE KINASE 2) (MAPKK 2) (ERK ACTIVATOR KINASE 2) (MAPK/ERK KINASE 2) (MEK2)
Q02750	DUAL SPECIFICITY MITOGEN-ACTIVATED PROTEIN KINASE KINASE 1 (MAP KINASE KINASE 1) (MAPKK 1) (ERK ACTIVATOR KINASE 1) (MAPK/ERK KINASE 1) (MEK1)

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Swiss-Prot Accession Number	Protein name
P49137	MAP KINASE-ACTIVATED PROTEIN KINASE 2 (MAPK-ACTIVATED PROTEIN KINASE 2) (MAPKAP KINASE 2) (MAPKAPK-2)
Q16539	MITOGEN-ACTIVATED PROTEIN KINASE 14 (MITOGEN-ACTIVATED PROTEIN KINASE P38) (MAP KINASE P38) (CYTOKINE SUPPRESSIVE ANTI-INFLAMMATORY DRUG BINDING PROTEIN) (CSAID BINDING PROTEIN) (CSBP) (MAX-INTERACTING PROTEIN 2) (MAP KINASE MXI2)
P53778	MITOGEN-ACTIVATED PROTEIN KINASE 12 (EXTRACELLULAR SIGNAL-REGULATED KINASE 6) (ERK-6) (ERK5) (STRESS-ACTIVATED PROTEIN KINASE-3) (MITOGEN-ACTIVATED PROTEIN KINASE P38 GAMMA) (MAP KINASE P38 GAMMA)
Q15759	MITOGEN-ACTIVATED PROTEIN KINASE 11 (MITOGEN-ACTIVATED PROTEIN KINASE P38 BETA) (MAP KINASE P38 BETA) (P38B) (P38-2) (STRESS-ACTIVATED PROTEIN KINASE-2)
P53779	MITOGEN-ACTIVATED PROTEIN KINASE 10 (STRESS-ACTIVATED PROTEIN KINASE JNK3) (C-JUN N-TERMINAL KINASE 3) (MAP KINASE P49 3F12)
P45984	MITOGEN-ACTIVATED PROTEIN KINASE 9 (STRESS-ACTIVATED PROTEIN KINASE JNK2) (C-JUN N-TERMINAL KINASE 2) (JNK-55)
P45983	MITOGEN-ACTIVATED PROTEIN KINASE 8 (STRESS-ACTIVATED PROTEIN KINASE JNK1) (C-JUN N-TERMINAL KINASE 1) (JNK-46)
Q13164	MITOGEN-ACTIVATED PROTEIN KINASE 7 (EXTRACELLULAR SIGNAL-REGULATED KINASE 5) (ERK-5) (ERK4) (BMK1 KINASE)
Q16659	MITOGEN-ACTIVATED PROTEIN KINASE 6 (EXTRACELLULAR SIGNAL-REGULATED KINASE 3) (ERK-3) (MAP KINASE ISOFORM P97) (P97-MAPK)
P31152	MITOGEN-ACTIVATED PROTEIN KINASE 4 (EXTRACELLULAR SIGNAL-REGULATED KINASE 4) (ERK-4) (MAP KINASE ISOFORM P63) (P63-MAPK)
P27361	MITOGEN-ACTIVATED PROTEIN KINASE 3 (EXTRACELLULAR SIGNAL-REGULATED KINASE 1) (ERK-1) (INSULIN-STIMULATED MAP2 KINASE) (MAP KINASE 1) (MAPK 1) (P44-ERK1) (ERT2) (P44-MAPK) (MICROTUBULE-ASSOCIATED PROTEIN-2 KINASE)
P28482	MITOGEN-ACTIVATED PROTEIN KINASE 1 (EXTRACELLULAR SIGNAL-REGULATED KINASE 2) (ERK-2) (MITOGEN-ACTIVATED PROTEIN KINASE 2) (MAP KINASE 2) (MAPK 2) (P42-MAPK) (ERT1)
Q12866	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE MER PRECURSOR (C-MER) (RECEPTOR TYROSINE KINASE MERTK)
P42679	MEGAKARYOCYTE-ASSOCIATED TYROSINE-PROTEIN KINASE (TYROSINE-PROTEIN KINASE CTK) (PROTEIN KINASE HYL) (HEMATOPOIETIC CONSENSUS TYROSINE-LACKING KINASE)
P20794	SERINE/THREONINE-PROTEIN KINASE MAK (MALE GERM CELL-ASSOCIATED KINASE)
P29966	MYRISTOYLATED ALANINE-RICH C-KINASE SUBSTRATE (MARCKS) (PROTEIN KINASE C SUBSTRATE, 80 KDA PROTEIN, LIGHT CHAIN) (PKCSL) (80K-L PROTEIN)
Q99558	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 14 (NF-KAPPA BETA-INDUCING KINASE) (SERINE/THREONINE PROTEIN KINASE NIK) (HSNIK)
Q02779	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 10 (MIXED LINEAGE KINASE 2) (PROTEIN KINASE MST)
P80192	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 9 (MIXED

[redacted]	[redacted]
	LINEAGE KINASE 1)
P41279	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 9 (COT PROTO-ONCOGENE SERINE/THREONINE-PROTEIN KINASE) (C-COT) (CANCER OSAKA THYROID ONCOGENE)
O43318	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 7 (TRANSFORMING GROWTH FACTOR-BETA-ACTIVATED KINASE 1) (TGF-BETA-ACTIVATED KINASE 1)
O95382	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 6
Q99683	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 5 (MAPK/ERK KINASE KINASE 5) (MEK KINASE 5) (MEKK 5) (APOPTOSIS SIGNAL-REGULATING KINASE 1) (ASK-1)
Q9Y6R4	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 4 (MAPK/ERK KINASE KINASE 4) (MEK KINASE 4) (MEKK 4) (MAP THREE KINASE 1)
Q99759	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 3 (MAPK/ERK KINASE KINASE 3) (MEK KINASE 3) (MEKK 3)
Q9Y2U5	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 2 (MAPK/ERK KINASE KINASE 2) (MEK KINASE 2) (MEKK 2)
Q13233	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 1 (MAPK/ERK KINASE KINASE 1) (MEK KINASE 1) (MEKK 1)
P07948	TYROSINE-PROTEIN KINASE LYN
P06239	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE LCK (P56-LCK) (LSK) (T CELL-SPECIFIC PROTEIN-TYROSINE KINASE)
P43405	TYROSINE-PROTEIN KINASE SYK (SPLEEN TYROSINE KINASE)
P08922	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE ROS PRECURSOR
P04049	RAF PROTO-ONCOGENE SERINE/THREONINE-PROTEIN KINASE (RAF-1) (C-RAF)
P31749	RAC-ALPHA SERINE/THREONINE KINASE (RAC-PK-ALPHA) (PROTEIN KINASE B) (PKB) (C-AKT)
P15056	B-RAF PROTO-ONCOGENE SERINE/THREONINE-PROTEIN KINASE (P94) (V-RAF MURINE SARCOMA VIRAL ONCOGENE HOMOLOG B1)
P10398	A-RAF PROTO-ONCOGENE SERINE/THREONINE-PROTEIN KINASE (ONCOGENE PKS2)
P14618	PYRUVATE KINASE, M1 ISOZYME (PYRUVATE KINASE MUSCLE ISOZYME) (CYTOSOLIC THYROID HORMONE-BINDING PROTEIN) (CTHBP) (THBP1)
Q07002	SERINE/THREONINE PROTEIN KINASE PCTAIRE-3
Q00537	SERINE/THREONINE-PROTEIN KINASE PCTAIRE-2
P11801	PUTATIVE SERINE/THREONINE-PROTEIN KINASE H1 (PSK-H1)
P11800	PUTATIVE SERINE/THREONINE-PROTEIN KINASE PSK-C3
P07557	PKS PROTO-ONCOGENE SERINE/THREONINE-PROTEIN KINASE (ONCOGENE PKS1)
Q05513	PROTEIN KINASE C, ZETA TYPE (NPKC-ZETA)
Q04759	PROTEIN KINASE C, THETA TYPE (NPKC-THETA)
O94806	PROTEIN KINASE C, NU TYPE (NPKC-NU) (PROTEIN KINASE EPK2)
Q15139	PROTEIN KINASE C, MU TYPE (NPKC-MU)
P24723	PROTEIN KINASE C, ETA TYPE (NPKC-ETA) (PKC-L)

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P41743	PROTEIN KINASE C, IOTA TYPE (NPKC-IOTA)
P05129	PROTEIN KINASE C, GAMMA TYPE (PKC-GAMMA)
Q02156	PROTEIN KINASE C, EPSILON TYPE (NPKC-EPSILON)
Q05655	PROTEIN KINASE C, DELTA TYPE (NPKC-DELTA)
P17252	PROTEIN KINASE C, ALPHA TYPE (PKC-ALPHA)
P05127	PROTEIN KINASE C, BETA-II TYPE (PKC-BETA-2)
P05771	PROTEIN KINASE C, BETA-I TYPE (PKC-BETA-1)
P27448	PUTATIVE SERINE/THREONINE-PROTEIN KINASE P78
P19525	INTERFERON-INDUCED, DOUBLE-STRANDED RNA-ACTIVATED PROTEIN KINASE (INTERFERON-INDUCIBLE RNA-DEPENDENT PROTEIN KINASE) (P68 KINASE) (P1/EIF-2A PROTEIN KINASE)
P21127	GALACTOSYLTRANSFERASE ASSOCIATED PROTEIN KINASE P58/GTA (CELL DIVISION CYCLE 2-LIKE 1) (CLK-1) (P58 CLK-1)
P00540	PROTO-ONCOGENE SERINE/THREONINE-PROTEIN KINASE MOS (C-MOS)
Q15746	MYOSIN LIGHT CHAIN KINASE, SMOOTH MUSCLE AND NON-MUSCLE ISOZYMES (MLCK) [CONTAINS: TELOKIN (KINASE RELATED PROTEIN) (KRP)]
P29376	LEUKOCYTE TYROSINE KINASE RECEPTOR PRECURSOR (PROTEIN TYROSINE KINASE-1)
P10721	MAST/STEM CELL GROWTH FACTOR RECEPTOR PRECURSOR (SCFR) (PROTO-ONCOGENE TYROSINE-PROTEIN KINASE KIT) (C-KIT) (CD117 ANTIGEN)
Q00532	SERINE/THREONINE-PROTEIN KINASE KKIALRE (CYCLIN-DEPENDENT KINASE-LIKE 1)
P37023	SERINE/THREONINE-PROTEIN KINASE RECEPTOR R3 PRECURSOR (SKR3) (ACTIVIN RECEPTOR-LIKE KINASE 1) (ALK-1) (TGF-B SUPERFAMILY RECEPTOR TYPE I) (TSR-I)
Q75838	KINASE INTERACTING PROTEIN 2 (KIP 2)
Q99828	DNA-PKCS INTERACTING PROTEIN (KINASE INTERACTING PROTEIN) (KIP) (CALCIUM AND INTEGRIN-BINDING PROTEIN) (CIB) (SNK INTERACTING PROTEIN 2-28) (SIP2-28)
P14619	CGMP-DEPENDENT PROTEIN KINASE 1, BETA ISOZYME (CGK 1 BETA) (CGKI-BETA)
Q13976	CGMP-DEPENDENT PROTEIN KINASE 1, ALPHA ISOZYME (CGK 1 ALPHA) (CGKI-ALPHA)
Q13237	CGMP-DEPENDENT PROTEIN KINASE 2 (CGK 2) (CGKII) (TYPE II CGMP-DEPENDENT PROTEIN KINASE)
Q13555	CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE TYPE II GAMMA CHAIN (CAM-KINASE II GAMMA CHAIN) (CAMK-II, GAMMA SUBUNIT)
Q13557	CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE TYPE II DELTA CHAIN (CAM-KINASE II DELTA CHAIN) (CAMK-II, DELTA SUBUNIT)
Q13554	CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE TYPE II BETA CHAIN (CAM-KINASE II BETA CHAIN) (CAMK-II, BETA SUBUNIT)
Q16566	CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE TYPE IV CATALYTIC CHAIN (CAM KINASE-GR) (CAMK IV) [CONTAINS: CALSPERMIN]
Q14012	CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE TYPE I (CAM

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	KINASE I)
P22612	CAMP-DEPENDENT PROTEIN KINASE, GAMMA-CATALYTIC SUBUNIT (PKA C-GAMMA)
P22694	CAMP-DEPENDENT PROTEIN KINASE, BETA-CATALYTIC SUBUNIT (PKA C-BETA)
P17612	CAMP-DEPENDENT PROTEIN KINASE, ALPHA-CATALYTIC SUBUNIT (PKA C-ALPHA)
P31323	CAMP-DEPENDENT PROTEIN KINASE TYPE II-BETA REGULATORY CHAIN
P13861	CAMP-DEPENDENT PROTEIN KINASE TYPE II-ALPHA REGULATORY CHAIN
P31321	CAMP-DEPENDENT PROTEIN KINASE TYPE I-BETA REGULATORY CHAIN
P10644	CAMP-DEPENDENT PROTEIN KINASE TYPE I-ALPHA REGULATORY CHAIN (TISSUE-SPECIFIC EXTINGUISHER-1) (TSE1)
Q9UBS0	RIBOSOMAL PROTEIN S6 KINASE BETA 2 (S6K-BETA 2) (70 KDA RIBOSOMAL PROTEIN S6 KINASE 2) (P70-S6KB) (P70 RIBOSOMAL S6 KINASE BETA) (P70 S6KBETA) (S6K2) (S6 KINASE-RELATED KINASE) (SRK) (SERINE/THREONINE-PROTEIN KINASE 14 BETA)
P23443	RIBOSOMAL PROTEIN S6 KINASE (S6K) (P70-S6K)
Q9UK32	RIBOSOMAL PROTEIN S6 KINASE ALPHA 6 (S6K-ALPHA 6) (90 KDA RIBOSOMAL PROTEIN S6 KINASE 6) (P90-RSK 6) (RIBOSOMAL S6 KINASE 4) (RSK-4) (PP90RSK4)
P51812	RIBOSOMAL PROTEIN S6 KINASE ALPHA 3 (S6K-ALPHA 3) (90 KDA RIBOSOMAL PROTEIN S6 KINASE 3) (P90-RSK 3) (RIBOSOMAL S6 KINASE 2) (RSK-2) (PP90RSK2) (INSULIN-STIMULATED PROTEIN KINASE 1) (ISPK-1)
Q15349	RIBOSOMAL PROTEIN S6 KINASE ALPHA 2 (S6K-ALPHA 2) (90 KDA RIBOSOMAL PROTEIN S6 KINASE 2) (P90-RSK 2) (RIBOSOMAL S6 KINASE 3) (RSK-3) (PP90RSK3)
Q15418	RIBOSOMAL PROTEIN S6 KINASE ALPHA 1 (S6K-ALPHA 1) (90 KDA RIBOSOMAL PROTEIN S6 KINASE 1) (P90-RSK 1) (RIBOSOMAL S6 KINASE 1) (RSK-1) (PP90RSK1)
P52333	TYROSINE-PROTEIN KINASE JAK3 (JANUS KINASE 3) (JAK-3) (LEUKOCYTE JANUS KINASE) (L-JAK)
P23458	TYROSINE-PROTEIN KINASE JAK1 (JANUS KINASE 1) (JAK-1)
Q08881	TYROSINE-PROTEIN KINASE ITK/TSK (T-CELL-SPECIFIC KINASE) (TYROSINE-PROTEIN KINASE LYK) (KINASE EMT)
Q9Y2B9	CAMP-DEPENDENT PROTEIN KINASE INHIBITOR, GAMMA FORM (PKI-GAMMA)
P04541	CAMP-DEPENDENT PROTEIN KINASE INHIBITOR, MUSCLE/BRAIN FORM (PKI-ALPHA)
P57043	INTEGRIN-LINKED PROTEIN KINASE 2 (ILK-2)
Q13418	INTEGRIN-LINKED PROTEIN KINASE 1 (ILK-1) (59 KDA SERINE/THREONINE PROTEIN KINASE) (P59ILK)
O95163	IKAPPAB KINASE COMPLEX-ASSOCIATED PROTEIN (IKK COMPLEX-ASSOCIATED PROTEIN) (P150)
P57058	HORMONALLY UPREGULATED NEU TUMOR-ASSOCIATED KINASE (SERINE/THREONINE PROTEIN KINASE MAK-V) (B19)
Q9UJY1	SMALL STRESS PROTEIN-LIKE PROTEIN HSP22 (E2IG1) (PROTEIN KINASE

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	H11)
Q9H2X6	HOMEODOMAIN-INTERACTING PROTEIN KINASE 2
P08631	TYROSINE-PROTEIN KINASE HCK (P59-HCK AND P60-HCK) (HEMOPOIETIC CELL KINASE)
P43250	G PROTEIN-COUPLED RECEPTOR KINASE GRK6
P34947	G PROTEIN-COUPLED RECEPTOR KINASE GRK5
P32298	G PROTEIN-COUPLED RECEPTOR KINASE GRK4 (ITI1)
Q14397	GLUCOKINASE REGULATORY PROTEIN (GLUCOKINASE REGULATOR)
P14314	PROTEIN KINASE C SUBSTRATE, 80 KD PROTEIN, HEAVY CHAIN (PKCSH) (80K-H PROTEIN)
P06241	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE FYN (P59-FYN) (SYN) (SLK)
P42685	TYROSINE-PROTEIN KINASE FRK (NUCLEAR TYROSINE PROTEIN KINASE RAK)
Q9HA64	HYPOTHETICAL FRUCTOSAMINE KINASE-LIKE PROTEIN FLJ12171/DKFZP564D202
P36888	FL CYTOKINE RECEPTOR PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR FLT3) (STEM CELL TYROSINE KINASE 1) (STK-1) (CD135 ANTIGEN)
P09769	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE FGR (P55-FGR) (C-FGR)
P07332	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE FES/FPS (C-FES)
P16591	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE FER (P94-FER) (C-FER)
Q05397	FOCAL ADHESION KINASE 1 (FADK 1) (PP125FAK) (PROTEIN-TYROSINE KINASE 2)
O15197	EPHRIN TYPE-B RECEPTOR 6 PRECURSOR (TYROSINE-PROTEIN KINASE-DEFECTIVE RECEPTOR EPH-6) (HEP)
P54760	EPHRIN TYPE-B RECEPTOR 4 PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR HTK)
Q15303	ERBB-4 RECEPTOR PROTEIN-TYROSINE KINASE PRECURSOR (P180ERBB4) (TYROSINE KINASE-TYPE CELL SURFACE RECEPTOR HER4)
P21860	ERBB-3 RECEPTOR PROTEIN-TYROSINE KINASE PRECURSOR (TYROSINE KINASE-TYPE CELL SURFACE RECEPTOR HER3)
P04626	RECEPTOR PROTEIN-TYROSINE KINASE ERBB-2 PRECURSOR (P185ERBB2) (NEU PROTO-ONCOGENE) (C-ERBB-2) (TYROSINE KINASE-TYPE CELL SURFACE RECEPTOR HER2) (MLN 19)
P54753	EPHRIN TYPE-B RECEPTOR 3 PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR HEK-2)
P29323	EPHRIN TYPE-B RECEPTOR 2 PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR EPH-3) (DRT) (RECEPTOR PROTEIN-TYROSINE KINASE HEK5) (ERK)
P54762	EPHRIN TYPE-B RECEPTOR 1 PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR EPH-2) (NET) (HEK6) (ELK)
P29322	EPHRIN TYPE-A RECEPTOR 8 (TYROSINE-PROTEIN KINASE RECEPTOR EEK) (EPH-AND ELK-RELATED KINASE) (HEK3)
Q15375	EPHRIN TYPE-A RECEPTOR 7 PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR EHK-3) (EPH HOMOLOGY KINASE-3) (RECEPTOR PROTEIN-

	TYROSINE KINASE HEK11)
P54756	EPHRIN TYPE-A RECEPTOR 5 PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR EHK-1) (EPH HOMOLOGY KINASE-1) (RECEPTOR PROTEIN-TYROSINE KINASE HEK7)
P54764	EPHRIN TYPE-A RECEPTOR 4 PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR SEK) (RECEPTOR PROTEIN-TYROSINE KINASE HEK8)
P29320	EPHRIN TYPE-A RECEPTOR 3 PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR ETK1) (HEK) (HEK4)
P29317	EPHRIN TYPE-A RECEPTOR 2 PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR ECK) (EPITHELIAL CELL KINASE)
P21709	EPHRIN TYPE-A RECEPTOR 1 PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR EPH)
Q9NVF9	ETHANOLAMINE KINASE-LIKE PROTEIN EKI2 (FLJ10761)
P20827	EPHRIN-A1 PRECURSOR (EPH-RELATED RECEPTOR TYROSINE KINASE LIGAND 1) (LERK-1) (IMMEDIATE EARLY RESPONSE PROTEIN B61) (TUMOR NECROSIS FACTOR, ALPHA-INDUCED PROTEIN 4)
Q99956	DUAL SPECIFICITY PROTEIN PHOSPHATASE 9 (MITOGEN-ACTIVATED PROTEIN KINASE PHOSPHATASE 4) (MAP KINASE PHOSPHATASE 4) (MKP-4)
Q16828	DUAL SPECIFICITY PROTEIN PHOSPHATASE 6 (MITOGEN-ACTIVATED PROTEIN KINASE PHOSPHATASE 3) (MAP KINASE PHOSPHATASE 3) (MKP-3) (DUAL SPECIFICITY PROTEIN PHOSPHATASE PYST1)
Q9Y463	DUAL-SPECIFICITY TYROSINE-PHOSPHORYLATION REGULATED KINASE 1B (MIRK PROTEIN KINASE)
Q13627	DUAL-SPECIFICITY TYROSINE-PHOSPHORYLATION REGULATED KINASE 1A (PROTEIN KINASE MINIBRAIN HOMOLOG) (MNBH) (HP86) (DUAL SPECIFICITY YAK1-RELATED KINASE)
P28562	DUAL SPECIFICITY PROTEIN PHOSPHATASE 1 (MAP KINASE PHOSPHATASE-1) (MKP-1) (PROTEIN-TYROSINE PHOSPHATASE CL100) (DUAL SPECIFICITY PROTEIN PHOSPHATASE HVH1)
Q09013	MYOTONIN-PROTEIN KINASE (MYOTONIC DYSTROPHY PROTEIN KINASE) (MDPK) (DM-KINASE) (DMK) (DMPK) (MT-PK)
Q16832	DISCOIDIN DOMAIN RECEPTOR 2 PRECURSOR (RECEPTOR PROTEIN-TYROSINE KINASE TKT) (TYROSINE-PROTEIN KINASE TYRO 10) (NEUROTROPHIC TYROSINE KINASE, RECEPTOR-RELATED 3)
Q08345	EPITHELIAL DISCOIDIN DOMAIN RECEPTOR 1 PRECURSOR (TYROSINE-PROTEIN KINASE CAK) (CELL ADHESION KINASE) (TYROSINE KINASE DDR) (DISCOIDIN RECEPTOR TYROSINE KINASE) (TRK E) (PROTEIN-TYROSINE KINASE RTK 6)
P53355	DEATH-ASSOCIATED PROTEIN KINASE 1 (DAP KINASE 1)
O15075	SERINE/THREONINE-PROTEIN KINASE DCAMKL1 (DOUBLECORTIN-LIKE AND CAM KINASE-LIKE 1)
P41240	TYROSINE-PROTEIN KINASE CSK (C-SRC KINASE) (PROTEIN-TYROSINE KINASE CYL)
Q9NYV4	CELL DIVISION CYCLE 2-RELATED PROTEIN KINASE 7 (CDC2-RELATED PROTEIN KINASE 7) (CRKRS)
Q9H4B4	CYTOKINE-INDUCIBLE SERINE/THREONINE-PROTEIN KINASE (FGF-

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	INDUCIBLE KINASE) (PROLIFERATION-RELATED KINASE)
P49761	PROTEIN KINASE CLK3
P49760	PROTEIN KINASE CLK2
P49759	PROTEIN KINASE CLK1 (CLK)
O96017	SERINE/THREONINE-PROTEIN KINASE CHK2 (CDS1)
O14757	SERINE/THREONINE-PROTEIN KINASE CHK1
Q16667	CYCLIN-DEPENDENT KINASE INHIBITOR 3 (CDK2-ASSOCIATED DUAL SPECIFICITY PHOSPHATASE) (KINASE ASSOCIATED PHOSPHATASE) (CYCLIN-DEPENDENT KINASE INTERACTING PROTEIN 2) (CYCLIN-DEPENDENT KINASE INTERACTOR 1)
Q14004	CELL DIVISION CYCLE 2-LIKE PROTEIN KINASE 5 (CHOLINESTERASE-RELATED CELL DIVISION CONTROLLER) (CDC2-RELATED PROTEIN KINASE 5)
Q15131	CELL DIVISION PROTEIN KINASE 10 (SERINE/THREONINE-PROTEIN KINASE PISSLRE)
P50750	CELL DIVISION PROTEIN KINASE 9 (SERINE/THREONINE-PROTEIN KINASE PITALRE) (C-2K)
P49336	CELL DIVISION PROTEIN KINASE 8 (PROTEIN KINASE K35)
P50613	CELL DIVISION PROTEIN KINASE 7 (CDK-ACTIVATING KINASE) (CAK) (39 KDA PROTEIN KINASE) (P39 MO15) (STK1) (CAK1)
Q00534	CELL DIVISION PROTEIN KINASE 6 (SERINE/THREONINE-PROTEIN KINASE PLSTIRE)
Q00535	CELL DIVISION PROTEIN KINASE 5 (TAU PROTEIN KINASE II CATALYTIC SUBUNIT) (TPKII CATALYTIC SUBUNIT) (SERINE/THREONINE-PROTEIN KINASE PSSALRE)
Q00526	CELL DIVISION PROTEIN KINASE 3
P24941	CELL DIVISION PROTEIN KINASE 2 (P33 PROTEIN KINASE)
O14519	CYCLIN-DEPENDENT KINASE 2-ASSOCIATED PROTEIN 1 (CDK2-ASSOCIATED PROTEIN 1) (PUTATIVE ORAL CANCER SUPPRESSOR) (DELETED IN ORAL CANCER-1) (DOC-1)
O00311	CELL DIVISION CYCLE 7-RELATED PROTEIN KINASE (CDC7-RELATED KINASE) (HSCDC7) (HUCDC7)
Q15078	CYCLIN-DEPENDENT KINASE 5 ACTIVATOR 1 PRECURSOR (CDK5 ACTIVATOR 1) (CYCLIN-DEPENDENT KINASE 5 REGULATORY SUBUNIT 1) (TAU PROTEIN KINASE II 23 KDA SUBUNIT) (TPKII REGULATORY SUBUNIT) (P23) (P25) (P35)
P06493	CELL DIVISION CONTROL PROTEIN 2 HOMOLOG (P34 PROTEIN KINASE) (CYCLIN-DEPENDENT KINASE 1) (CDK1)
Q9UHJ6	CARBOHYDRATE KINASE-LIKE PROTEIN
O43683	MITOTIC CHECKPOINT SERINE/THREONINE-PROTEIN KINASE BUB1 (HBUB1) (BUB1A)
O60566	MITOTIC CHECKPOINT SERINE/THREONINE-PROTEIN KINASE BUB1 BETA (HBUBR1) (MAD3/BUB1-RELATED PROTEIN KINASE) (MITOTIC CHECKPOINT KINASE MAD3L)
Q06187	TYROSINE-PROTEIN KINASE BTK (BRUTON'S TYROSINE KINASE) (AGAMMAGLOBULINAEMIA TYROSINE KINASE) (ATK) (B CELL

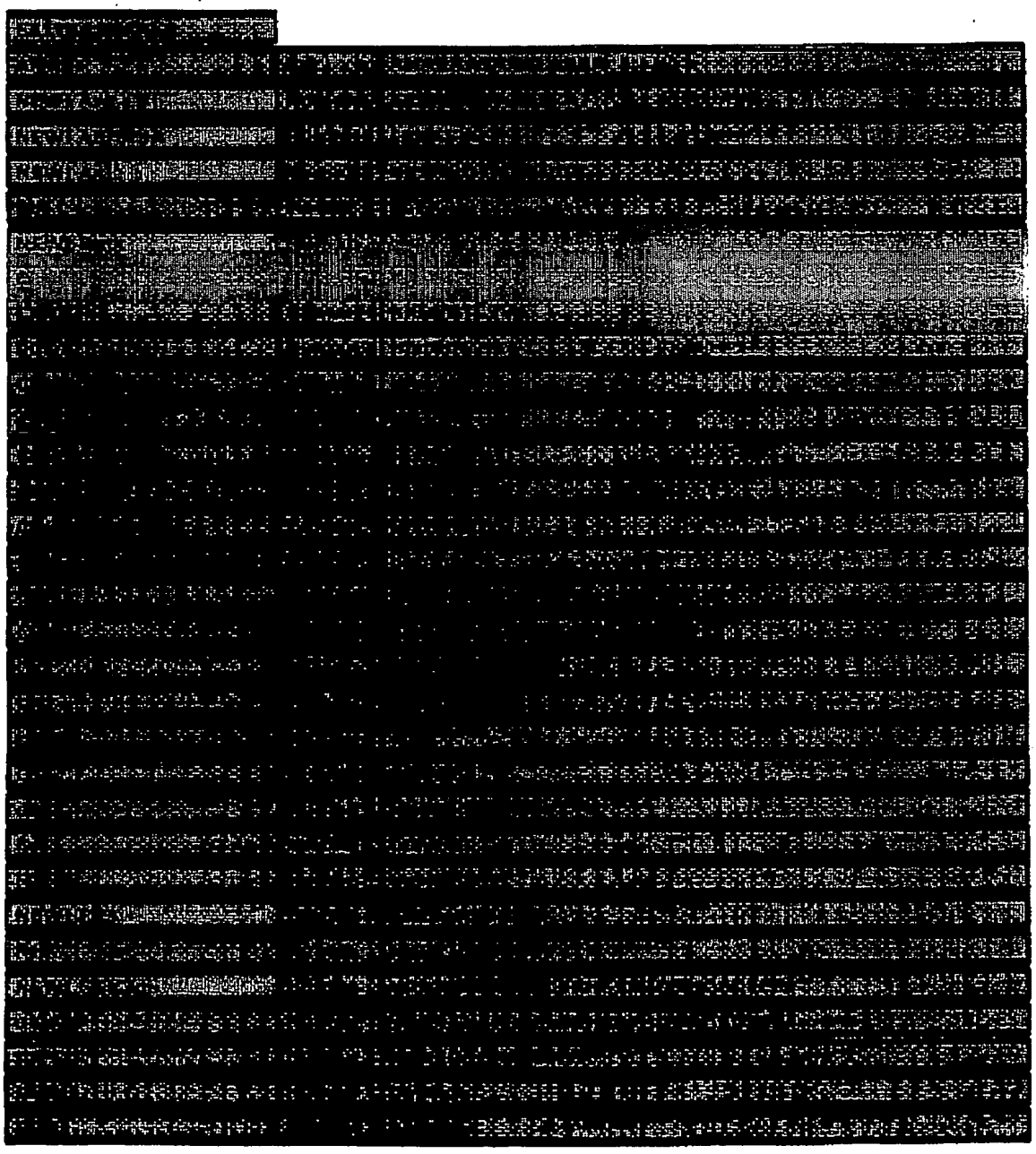
	PROGENITOR KINASE) (BPK)
P51813	CYTOPLASMIC TYROSINE-PROTEIN KINASE BMX (BONE MARROW KINASE BMX) (EPITHELIAL AND ENDOTHELIAL TYROSINE KINASE) (ETK) (NTK38)
P36894	BONE MORPHOGENETIC PROTEIN RECEPTOR TYPE IA PRECURSOR (SERINE/THREONINE-PROTEIN KINASE RECEPTOR R5) (SKR5) (ACTIVIN RECEPTOR-LIKE KINASE 3) (ALK-3)
P51451	TYROSINE-PROTEIN KINASE BLK (B LYMPHOCYTE KINASE) (P55-BLK)
Q04771	ACTIVIN RECEPTOR TYPE I PRECURSOR (ACTR-I) (SERINE/THREONINE-PROTEIN KINASE RECEPTOR R1) (SKR1) (ACTIVIN RECEPTOR-LIKE KINASE 2) (ALK-2) (TGF-B SUPERFAMILY RECEPTOR TYPE I) (TSR-I)
Q13315	SERINE-PROTEIN KINASE ATM (ATAXIA TELANGIECTASIA MUTATED) (A-T, MUTATED)
P35626	BETA-ADRENERGIC RECEPTOR KINASE 2 (BETA-ARK-2) (G-PROTEIN COUPLED RECEPTOR KINASE 3)
P25098	BETA-ADRENERGIC RECEPTOR KINASE 1 (BETA-ARK-1) (G-PROTEIN COUPLED RECEPTOR KINASE 2)
P57078	SERINE/THREONINE-PROTEIN KINASE ANKRD3 (ANKYRIN REPEAT DOMAIN PROTEIN 3)
Q9Y243	RAC-GAMMA SERINE/THREONINE PROTEIN KINASE (RAC-PK-GAMMA) (PROTEIN KINASE AKT-3) (PROTEIN KINASE B, GAMMA) (PKB GAMMA)
P31751	RAC-BETA SERINE/THREONINE PROTEIN KINASE (RAC-PK-BETA) (PROTEIN KINASE AKT-2) (PROTEIN KINASE B, BETA) (PKB BETA)
Q02952	A-KINASE ANCHOR PROTEIN 12 (A-KINASE ANCHOR PROTEIN 250 KDA) (AKAP 250) (MYASTHENIA GRAVIS AUTOANTIGEN GRAVIN)
Q99996	A KINASE ANCHOR PROTEIN 9 (PROTEIN KINASE A ANCHORING PROTEIN 9) (PRKA9) (A-KINASE ANCHOR PROTEIN 450 KDA) (AKAP 450) (A-KINASE ANCHOR PROTEIN 350 KDA) (AKAP 350) (HGAKAP 350) (AKAP 120 LIKE PROTEIN) (HYPERION PROTEIN) (YOTIAO PROTEIN) (CENTROS
O43823	A-KINASE ANCHOR PROTEIN 8 (A-KINASE ANCHOR PROTEIN 95 KDA) (AKAP 95)
O43687	A-KINASE ANCHOR PROTEIN 7 (A-KINASE ANCHOR PROTEIN 9 KDA)
Q13023	A KINASE ANCHOR PROTEIN 6 (PROTEIN KINASE A ANCHORING PROTEIN 6) (PRKA6) (A-KINASE ANCHOR PROTEIN 100 KDA) (AKAP 100) (MAKAP)
P24588	A-KINASE ANCHOR PROTEIN 5 (A-KINASE ANCHOR PROTEIN 79 KDA) (AKAP 79) (CAMP-DEPENDENT PROTEIN KINASE REGULATORY SUBUNIT II HIGH AFFINITY BINDING PROTEIN) (H21)
O75969	A KINASE ANCHOR PROTEIN 3 (PROTEIN KINASE A ANCHORING PROTEIN 3) (PRKA3) (A-KINASE ANCHOR PROTEIN 110 KDA) (AKAP 110) (SPERM OOCYTE BINDING PROTEIN) (FIBROUSHEATHIN I) (FIBROUS SHEATH PROTEIN OF 95 KDA) (FSP95)
Q9Y2D5	A KINASE ANCHOR PROTEIN 2 (PROTEIN KINASE A ANCHORING PROTEIN 2) (PRKA2)
Q92667	A KINASE ANCHOR PROTEIN 1, MITOCHONDRIAL PRECURSOR (PROTEIN KINASE A ANCHORING PROTEIN 1) (PRKA1) (A-KINASE ANCHOR PROTEIN 149 KDA) (AKAP 149) (DUAL SPECIFICITY A-KINASE ANCHORING PROTEIN 1) (D-AKAP-1) (SPERMATID A-KINASE ANCHOR PROTEIN 84) (S-
Q9UKA4	A KINASE ANCHOR PROTEIN 11 (PROTEIN KINASE A ANCHORING

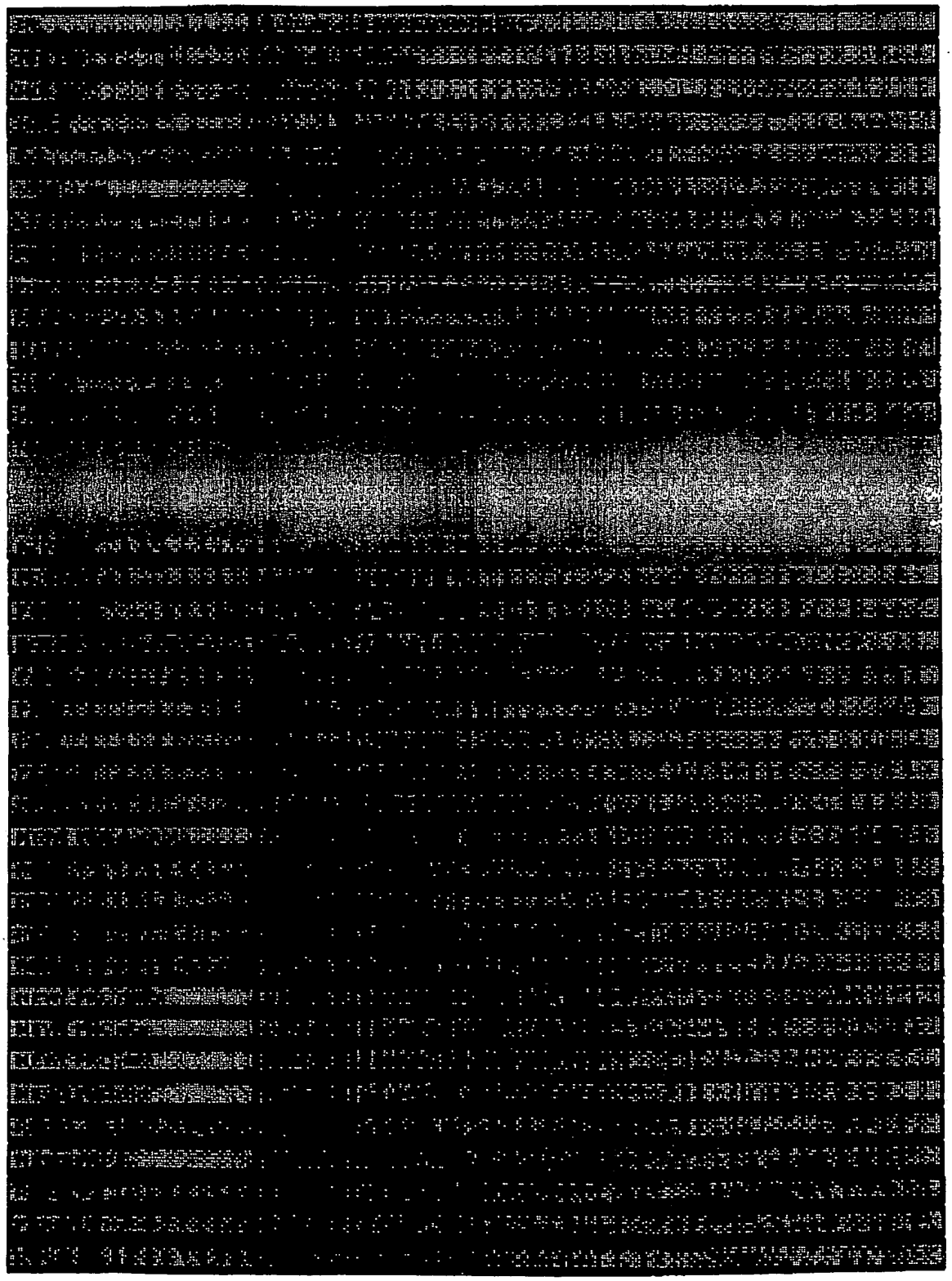
[illegible]	[illegible]
	PROTEIN 11) (PRKA11) (A KINASE ANCHOR PROTEIN 220 KDA) (AKAP 220) (HAKAP220)
O43572	A KINASE ANCHOR PROTEIN 10, MITOCHONDRIAL PRECURSOR (PROTEIN KINASE A ANCHORING PROTEIN 10) (PRKA10) (DUAL SPECIFICITY A-KINASE ANCHORING PROTEIN 2) (D-AKAP-2)
P42684	TYROSINE-PROTEIN KINASE ABL2 (TYROSINE KINASE ARG)
P00519	PROTO-ONCOGENE TYROSINE-PROTEIN KINASE ABL1 (P150) (C-ABL)
Q9UGI9	5'-AMP-ACTIVATED PROTEIN KINASE, GAMMA-3 SUBUNIT (AMPK GAMMA-3 CHAIN) (AMPK GAMMA3)
Q9UGJ0	5'-AMP-ACTIVATED PROTEIN KINASE, GAMMA-2 SUBUNIT (AMPK GAMMA-2 CHAIN) (AMPK GAMMA2) (H91620P)
P54619	5'-AMP-ACTIVATED PROTEIN KINASE, GAMMA-1 SUBUNIT (AMPK GAMMA-1 CHAIN) (AMPKG)
O43741	5'-AMP-ACTIVATED PROTEIN KINASE, BETA-2 SUBUNIT (AMPK BETA-2 CHAIN)
Q9Y478	5'-AMP-ACTIVATED PROTEIN KINASE, BETA-1 SUBUNIT (AMPK BETA-1 CHAIN) (AMPKB)
P54646	5'-AMP-ACTIVATED PROTEIN KINASE, CATALYTIC ALPHA-2 CHAIN (AMPK ALPHA-2 CHAIN)
Q13131	5'-AMP-ACTIVATED PROTEIN KINASE, CATALYTIC ALPHA-1 CHAIN (AMPK ALPHA-1 CHAIN)
P42655	14-3-3 PROTEIN EPSILON (MITOCHONDRIAL IMPORT STIMULATION FACTOR L SUBUNIT) (PROTEIN KINASE C INHIBITOR PROTEIN-1) (KCIP-1) (14-3-3E)
P31946	14-3-3 PROTEIN BETA/ALPHA (PROTEIN KINASE C INHIBITOR PROTEIN-1) (KCIP-1) (PROTEIN 1054)

Table 2

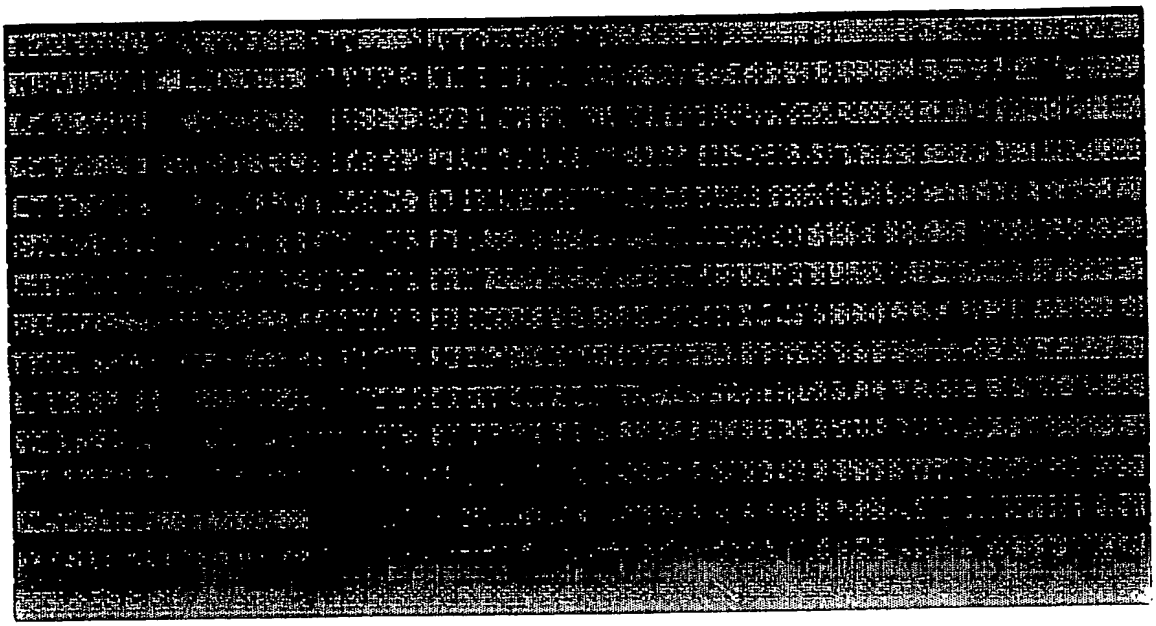
This is a list of substrates as available from Upstate.

http://www.upstate.com/features/kinaseprofiler.q./KinaseProfiler+%23153%3B#Kinase1





P1660



ABSTRACT

The present invention relates to an enzyme array and assay for use with a mass spectrometer, particularly, though not exclusively, a laser desorption/ ionisation, such as a MALDI mass spectrometer. It includes a method of determining the activity of an enzyme, or the effect a test compound has on the activity of the enzyme, using mass spectrometry comprising:providing a probe carrying an immobilised enzyme;optionally introducing the test compound;introducing one or more reactants to the immobilised enzyme for a time, and in a form sufficient for a reaction to take place; drying the probe; subjecting the probe to mass spectrometry; and determining the activity of the enzyme, or the effect the test compound had on the activity of the enzyme, by detecting the presence and/ or absence of one or more products and/ or the one or more reactants and is exemplified with reference to a kinase assay. It also provides an array for use with the method.

Fig 3.

Coupling Procedures for proteins on surfaces
Random coupling
Ordered coupling
BCCP-fusion-biotin
Neutravidin
PEG-PLL-biotin
Amine coupling
PEG-PLL

FIG. 1 (1/3)

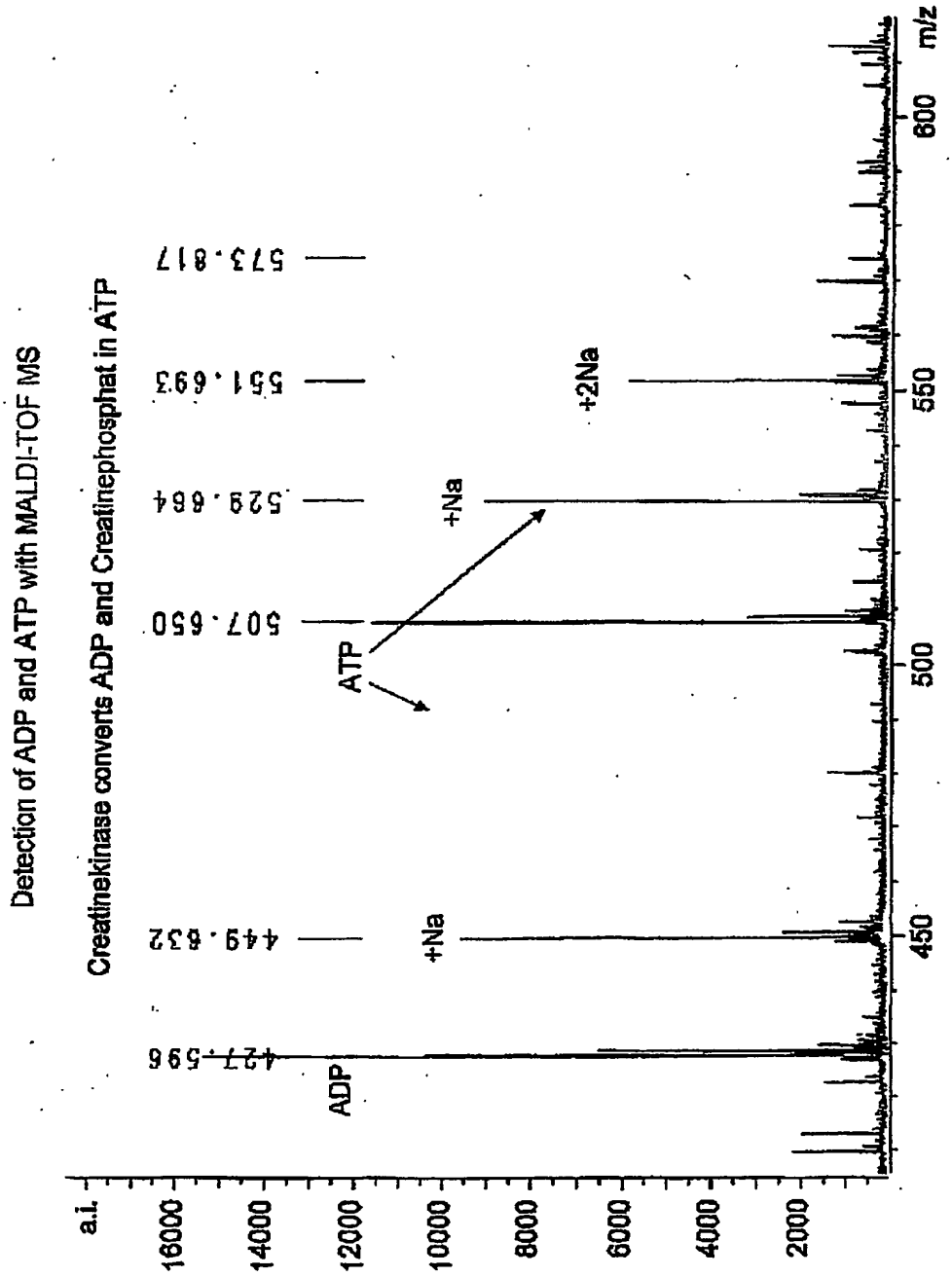

Detection of ADP and ATP with MALDI-TOF MS
Creatinekinase converts ADP and Creatinephosphat in ATP
a.i.
16000
14000
12000
10000
8000
6000
4000
2000
m/z
600
550
500
450
573.817
551.693
529.664
507.650
449.632
427.596
ADP
ATP
+Na
+Na
+2Na

FIG. 2 (2/3)

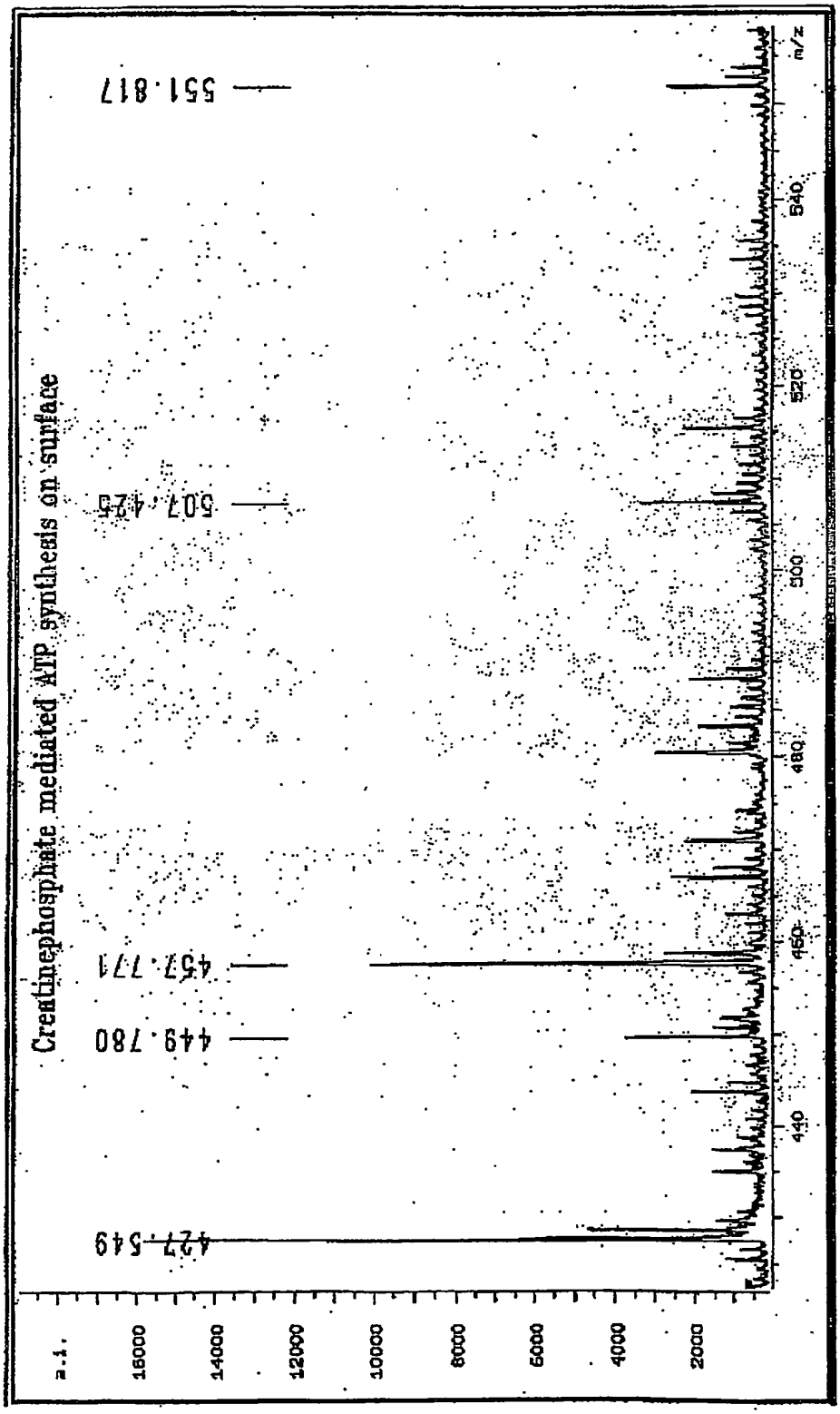
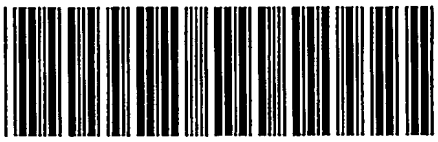

Creatinephosphate mediated ATP synthesis on surface
427.549
449.780
457.771
507.425
551.817
a.i.
16000
14000
12000
10000
8000
6000
4000
2000
440
460
480
500
520
540
m/z

FIG. 3 (3/3)

PCT Application

IB0305427



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